“Comparative phytochemical study of heartwood versus small branches of *acacia catechu* using high performance thin layer chromatography(HPTLC)”

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Central Council for Research in Ayurvedic Sciences

It is an apex body
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development and
promotion
of research
on scientific lines
in Ayurveda systems
of medicine

- Basic Research
- Clinical Research
- Drug Research
- Literature Research
- New Drug Development
- Collaborative Research Studies/ Research Collaborations
- Research Database

TYPES OF ISM DRUGS

PLANTS / ANIMAL /MINERAL ORIGIN

Forms to be uses

- **Raw drugs(Plants)-**
- **Mineral / Metal drugs/Animal origin**
- **Bhasmas / Raskalpas**
- **Single / Polyherbal formulation**
- **Herbo-mineral/ metallic preparations**

- ✓ **Ayurvedic Pharmacopoeial of India[API] approx. 600 Single Drugs.**
  - ✓ **Single Plant Drugs----** 90-95 %()

-Root	-Flower
-Stem	-Fruit
-Leaf	-Seed

- ➢ Roots/Root bark of plants as drug:- **20%**

Introduction:

- There are several plants that are being used traditionally for preparation of Ayurvedic/Herbal drugs.
  - ✓ Wild source
  - ✓ By cultivation
- The conservation of these plants species on long term basis is also questionable. It is a fact that there is not enough realization/awareness even among the users and stockholders that several of the wild medicinal plant species are threatened with extinction.
- The root and stem bark are being used in the formulations related to the Ayurveda. To prepare these formulations, it becomes imperative to disturb the tree or whole plant for collection of root/stem bark. On such circumstances these plants would become extinct.

- The Forest Department has laid down stringent rule not to collect the above from the forest area. Thus the availability of such material are becoming rare.
- However in their name other similar Raw drugs which are available in the market are being termed as its substitutes/adulterants.

✓ To over come this problem and to help the manufacturer's it is essential to study the **Phytochemical profile of root/root bark/stem bark** of the plant and be compared with the **Aerial part/leaves** of the same plant in order to save the plants without much disturbance. This study would thus pave way for a better use of herbs available without disturbing the plant from which the parts are derived. The many plants have been chosen for the above study.

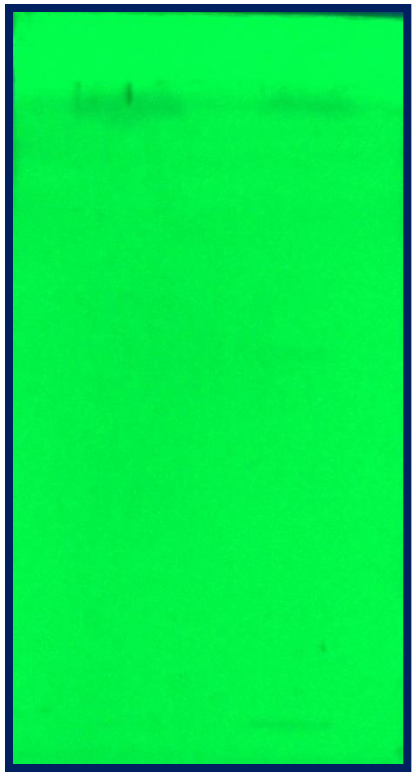
Objective:

To compare the phyto-constituents present in the heartwood and small branches of *Acacia catechu* on the basis of high performance thin layer chromatography (HPTLC) in order to evaluate whether the small branches of this plant may be substituted with the heartwood(official part). *Acacia catechu* (Family- Mimosaceae) commonly called Khadira is a medicinal plant widely used in Ayurveda. As per the Ayurvedic literature, heartwood of this plant is used in sáotha, Kustha, Prameha, Vrana· It is difficult to get huge amount of heartwood from this big tree without cutting the plant. Due to which availability of this plant may be difficult in near future for use in Indian system of medicine.

Methods:

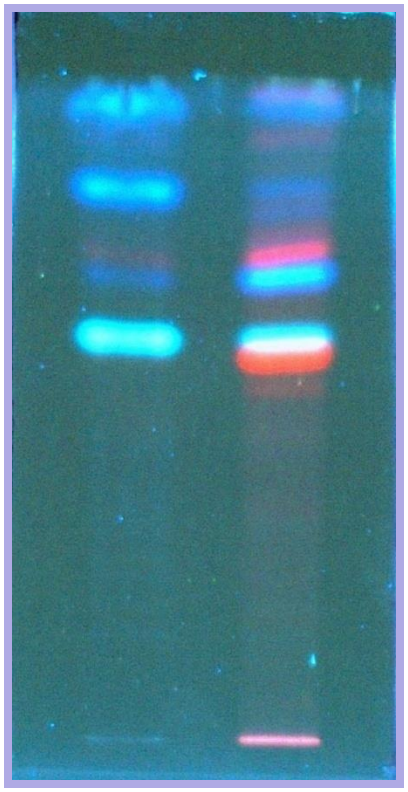
- CAMAG HPTLC system equipped with semi-automatic applicator Linomat-IV and win CATS 1.4.2 software was used.
- *n*-hexane, ethyl acetate and ethanolic extracts of the heartwood and small braches were prepared.
- And developed in suitable mobile phase(*Toluene: Ethyl acetate (7:3 v/v)*) using standard procedures and visualized in UV 254 and 366 nm and in white light after derivatization with in Anisaldehyde-Sulphuric acid reagent.

1. Using *n*- hexane extractive



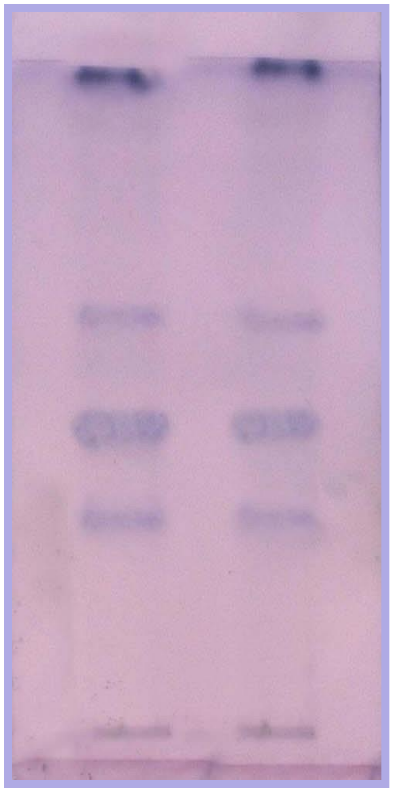
1 2

254 nm



1 2

366 nm



1 2

After derivatization

Observations:

TLC of *n*- hexane extracts of Heartwood and Small branches (stem) of Acacia Catechu

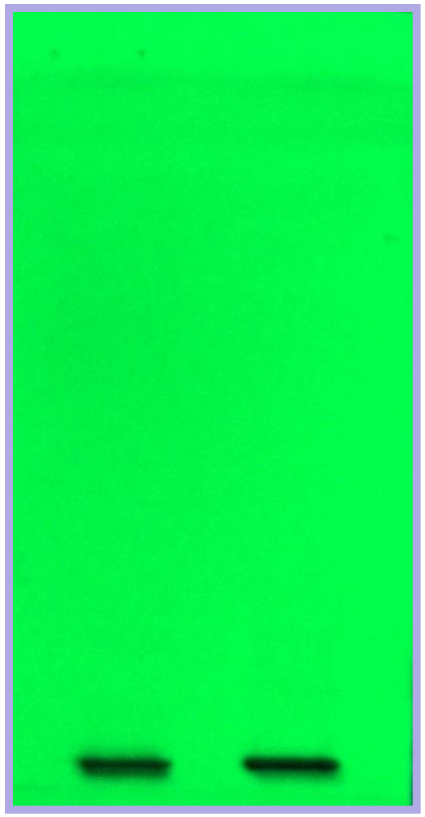
Solvent system: Toluene: Ethyl acetate (7:3 v/v)

Track 1: Heartwood - 10 µl

Track 2: Small branches (stem) - 10 µl

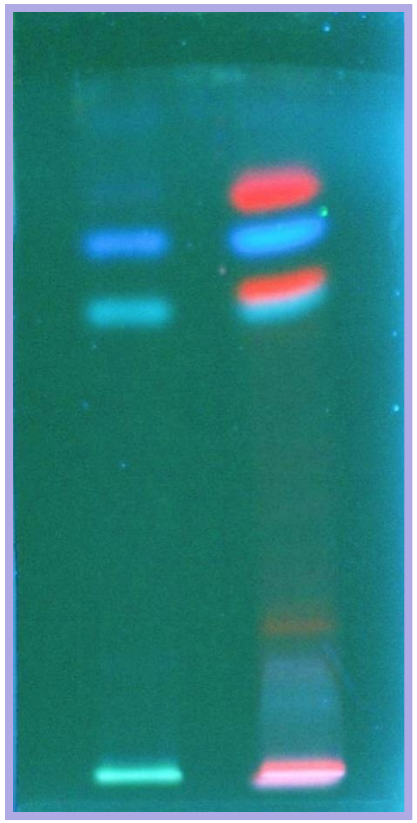
Track	UV 254 nm	UV 366 nm	After derivatization
Track 1	No band	0.61 (florescent blue), 0.69 (blue), 0.73 (red), 0.82 (florescent blue), 0.95 (florescent blue)	0.38 (blue), 0.54 (blue), 0.68 (blue), 0.94 (blue)
Track 2	No band	0.61 (red & florescent blue), 0.69 (florescent blue), 0.73 (red), 0.82 (red), 0.95 (blue) ➢ 5 bands are same	0.38 (blue), 0.54 (blue), 0.68 (blue), 0.94 (blue) ➢ 4 bands are same

2. Using ethyl acetate extractive



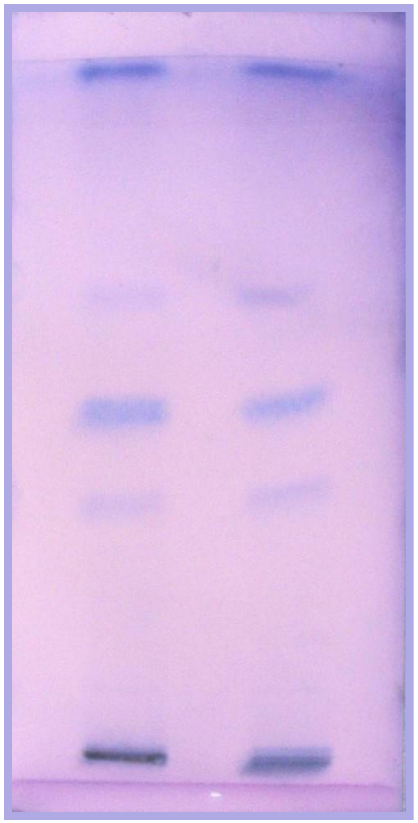
1 2

254 nm



1 2

366 nm



1 2

After derivatization

Observations:

TLC of ethyl acetate extracts of Heartwood and Small branches (stem) of *Acacia Catechu*

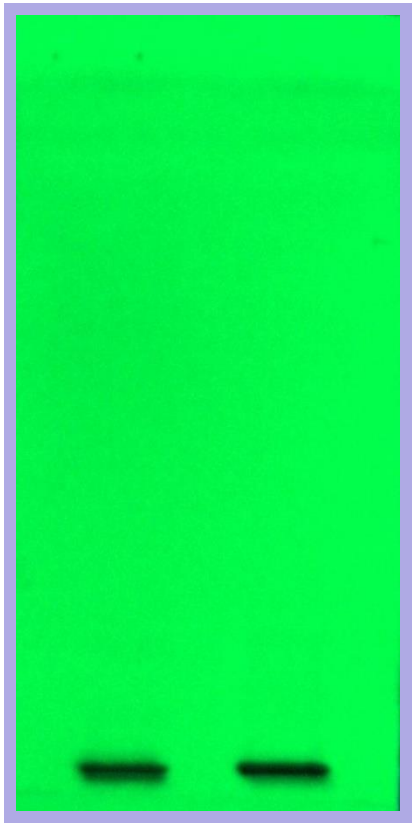
Solvent system: *Toluene: Ethyl acetate* (7:3 v/v)

Track 1: Heartwood - 10 µl

Track 2: Small branches (stem) - 10 µl

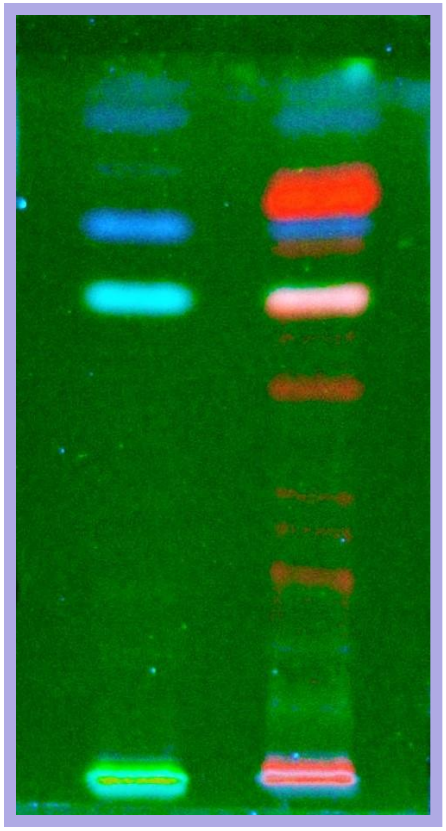
Track	UV 254 nm	UV 366 nm	After derivatization
Track 1	No band	0.66 (green), 0.74 (blue)	0.40 (blue), 0.52 (blue), 0.67 (blue), 0.98 (blue)
Track 2	No band	0.23 (red), 0.66 (green), 0.70 (red), 0.74 (blue), 0.82 (red) ➢2 bands are same	0.40 (blue), 0.52 (blue), 0.67 (blue), 0.98 (blue) ➢4 bands are same

3. Using Alcohol extract



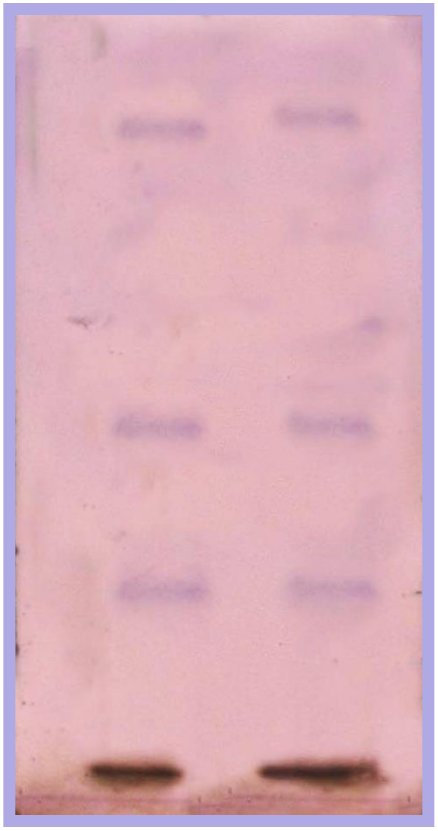
` 1 2

254 nm



1 2

366 nm



1 2

After derivatization

Observations:

TLC of ethyl acetate extracts of Heartwood and Small branches (stem) of *Acacia Catechu*

Solvent system: *Toluene: Ethyl acetate (7:3 v/v)*

Track 1: Heartwood - 10 µl

Track 2: Small branches (stem) - 10 µl

Track	UV 254 nm	UV 366 nm	After derivatization
Track 1	No band	0.66 (florescent green), 0.74 (blue), 0.98 (blue)	0.38 (blue), 0.52 (blue), 0.97 (blue)
Track 2	No band	0.23 (red), 0.66 (green), 0.70 (red), 0.74 (blue), 0.82 (red), 0.98 (blue) ➢3 bands are same	0.38 (blue), 0.52 (blue), 0.97 (blue) ➢3 bands are same

Conclusion:

The HPTLC fingerprinting of the *n*-hexane, ethyl acetate and ethanolic extracts of heartwood and small branches showed almost similar phytochemical profile, therefore small branches may be used in place of heartwood (official part) and vice-versa after comparison and confirmation of same for pharmacological activities.

The results of qualitative evaluation of HPTLC fingerprint profile will also be helpful in the identification and quality control of the drug and can provide standard HPTLC fingerprints with selected solvent system. The method can also be used for identification of different *Acacia catechu* species and adulterants.


Thank
You